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Genetic Variations Study in Polymorphism of VEGF 936 C/T Gene and the Immunological Studies Include TORCH Infections In Repeated Abortion.

[1] N.Ashok Vardhan, [2] Jyothsna Jaladi, [1] Prof. A.Rojarani*
[1]Department of Genetics Osmania University, Hyderabad & [2]Department of Biotechnology SRM University, Potheri, Tamilnadu-603203.

Abstract: *Repeated abortion, Habitual abortion, recurrent miscarriage or recurrent pregnancy loss (RPL) is the occurrence of three or more Pregnancies that end in miscarriage of the fetus, usually before 20 weeks of gestation. RPL affects 0.34% of women who conceive. When miscarriage occurs repeatedly at a certain period of pregnancy, it is termed `habitual abortion`. Repeated abortion takes place due to various causes like chromosomal abnormalities, physical illness, polycystic ovary syndrome, immune problems, antiphospholipid antibodies, problems in the uterus, intra uterine natural killer cells, thrombophilia, and life style in women. And some more valid issues that we have performed a study on genetic and immunological aspects like pathophysiology, angiogenesis, VEGF gene polymorphism, IgG, IgM And TORCH (which stands for Toxoplasmosis, Rubella, Cytomegalovirus and Herpes) . In this study we have taken 60 numbers of female patients in the age group between 24±6 age, by using techniques like agarose gel electrophoresis, PCR, RFLP and TORCH tests were performed in the sterile laboratory conditions to get accurate results in the patient samples. We determined IgG and IgM levels specific of TORCH group of infectious agents in women with recurrent miscarriage.*

Keywords: *Repeated abortion, Habitual abortion, recurrent miscarriage, VEGF gene polymorphism and TORCH infection*

I. Introduction

The term abortion is commonly also known as miscarriage and it simply means the expulsion of the fetus from the uterus before the complete formation of the placenta. This may occur any time before 28 weeks of gestation but is most common during the first 12 weeks of pregnancy. One in five to ten pregnancies terminates in this way. However, when miscarriage occurs repeatedly at a certain period of pregnancy, it is termed `habitual abortion` (Eastman, N. J. 1947). It is one of the most perplexing problems of gynecology and a major cause of maternal mortality. A woman who has suffered two or more terminations of this sort consecutively is said to be a case of habitual abortion. Depending upon the age and health of the pregnant woman. Most miscarriages occur very early in pregnancy in most cases they occur so early in the pregnancy that the woman is not even aware that she was pregnant. Statistically RPL affects 0.34% of women who conceive.

The most common cause of spontaneous abortion during the first trimester (Cnattingius S et al 2000) is chromosomal abnormalities and infections of the embryo/fetus, accounting for at least 50% of sampled early pregnancy losses.(Maldonado YA et al; 2011) Repeated abortion takes place due to various causes like chromosomal abnormalities, physical illness, polycystic ovary syndrome, immune problems, antiphospholipid antibodies, problems in the uterus, intra uterine natural killer cells (Moffett et al.), thrombophilia, life style in women. Some side effects cause of smoking (Nes RB et.al; 1993) and alcohol consumption. (Abel E, 1997)

II. Materials And Methods

A total of the 120 women were selected from Modern Maternity Hospital, Petalburz, Institute of Genetics and Hospital for Genetic Diseases and SRM Medical college Hospital and Research center. Out of 120 sixty were diseased and remaining 60 were normal. The patients were informed about the investigations and the detailed clinical history of the patients was sought after the permission grant from the hospital superintendent. 5ml of venous blood was collected in which 3ml was collected in to EDTA vacutainers and was stored in -20^0C in EDTA tubes for DNA isolation and remaining 2ml was collected in collecting tubules for Torch test. Then isolation of genomic DNA by spin column kit method, agarose gel electrophoresis, polymerase chain reaction (PCR), RFLP and TORCH tests were carried with respective standard protocols to obtain the results.

Factors affects in RPL:
Etiological factors: defective cogenesis or spermatogenesis; thyroid hypo function; serologic disturbances; vitamin deficiencies (especially of vitamin E, C and K) disturbances of glycogen metabolism and storage in the endometrial; disturbances of anterior pituitary and corpus luteum; and disturbances in estrogen production

Pathophysiology – The fetal or placental defect or the maternal condition results in the disruption of blood flow, containing oxygen and nutrients, to the developing fetus. The fetus is compromised and subsequently expelled from the uterus

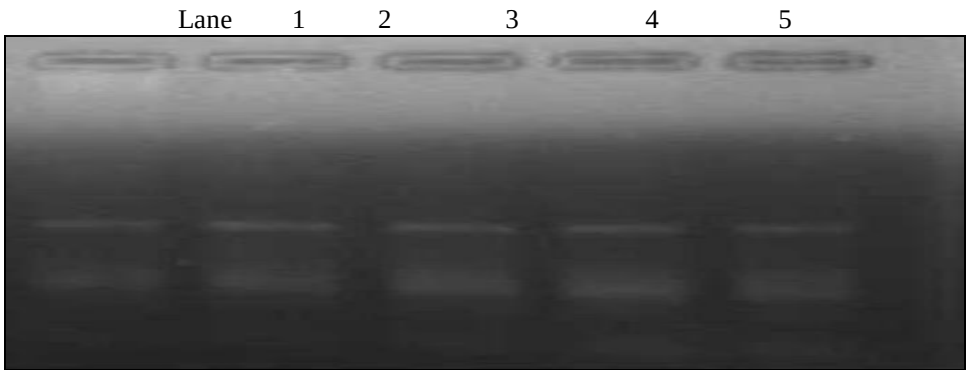
Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels. (John S. Penn 11 March 2008). Angiogenesis is a normal and vital process in growth and development, as well as in wound healing and in granulation tissue (Zygmunt M et.al 2003). This neovascular mechanism is mediated by the vascular endothelial growth factor (VEGF) family of cytokines.
The corpus luteum (CL) is a site of intense angiogenesis, the formation of a dense capillary network enabling the hormone-producing cells to obtain the oxygen, nutrients and hormone precursors necessary to synthesize and release large amounts of progesterone required for establishment and maintenance of early pregnancy (Gaytán F, Morales C, García-Pardo L,)

Vascular endothelial growth factor (**VEGF**) is a signal protein produced by cells that stimulates the growth of new blood vessels. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. VEGF's normal function is to create new blood vessels during embryonic development, (Goto F, Goto November 1993) new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels. There are sub types of VEGF also in their family (Li X, Eriksson U, 2001)

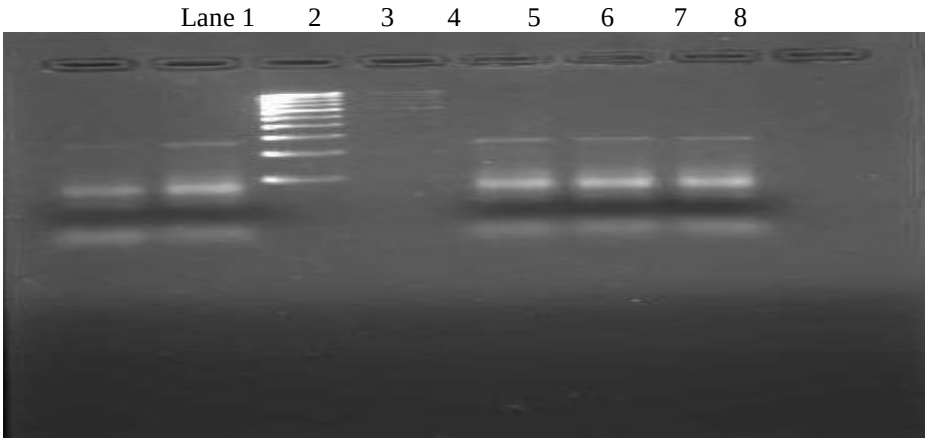
III. Results

Out of 60 women with Repeated Abortion studied, there is increased proportion of 936CC in 48cases (80%), whereas 936CT was found in 12 cases (20 %). The results are depicted in the below table:

	VEGF 936CC	VEGF936 CT
Repeatedly Aborted Women	80(%)	20(%)



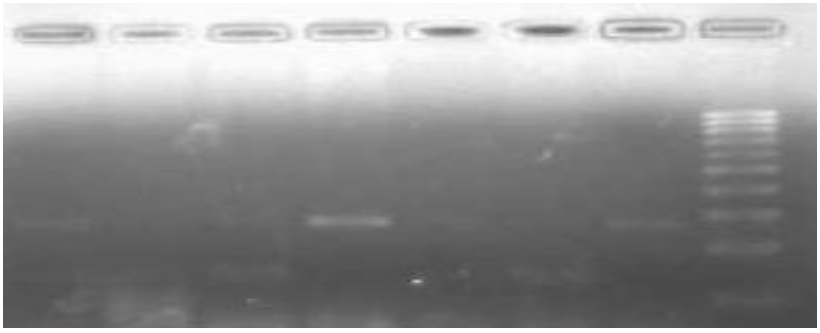
Agarose Gel Electrophoresis for VEGF 936 C/T PCR product of 266bp



Agarose Gel Electrophoresis for VEGF 936 C/T

The PCR products of 266bp were digested by endonuclease NlaIII; the 936T allele gave two fragments of 210 and 56 bp while the 936C allele remained uncut. Lane 3- DNA marker of 100bp; lanes 1, 2,5,6,7 - 936CC (wild type homozygous genotype).

Lane 1 2 3 4 5 6 7 8



Agarose Gel Electrophoresis for VEGF 936 C/T

The PCR products of 266bp were digested by endonuclease NlaIII; the 936T allele gave two fragments of 210 and 56 bp while the 936C allele remained uncut. Lane 8- DNA marker of 100bp; lanes 1-3 936CC (wild type homozygous genotype); lane 4,7 are, 936T (heterozygous genotype).

Torch Results:

IgG**ID OF THE PATIENT**	**TOXOPLASMOSIS**	**RUBELLA**	**CYTOMEGALO VIRUS**	**HERPES SIMPLEX VIRUS**
730208	0.32	1.58	2.07	4.32
730209	0.47	4.55	2.45	3.71
730210	0.22	3.62	3.95	4.47
730211	0.21	3.21	2.17	3.33
730212	2.45	1.34	2.56	3.09
730213	0.20	0.21	1.45	2.90
730214	0.19	0.11	2.60	1.97
730215	0.20	5.42	3.60	0.27
730216	0.15	1.03	3.11	2.16
730217	0.20	4.72	1.76	3.80
730218	0.17	0.48	2.15	2.24
730219	0.20	2.76	1.28	1.64
730220	0.24	3.67	2.53	3.43
730221	0.42	5.81	7.30	0.26
730222	0.34	4.10	3.51	0.26
730223	0.24	5.17	6.89	5.81
730224	0.26	2.53	3.03	1.88
730225	3.90	5.25	2.57	2.27
730226	1.09	0.43	1.65	1.54
730227	0.57	6.7	5.60	0.23

IgM

ID OF THE PATIENT	**TOXOPLASMOSIS**	**RUBELLA**	**CYTOMEGALO VIRUS**	**HERPES SIMPLEX VIRUS**
730208	0.45	0.65	0.23	0.97
730209	0.45	0.36	0.74	0.41
730210	0.26	0.30	0.36	0.39
730211	0.49	0.40	0.42	0.26
730212	0.34	0.19	0.29	0.85
730213	0.29	o.31	o.43	0.29
730214	0.68	0.07	0.23	0.20
730215	0.98	0.91	1.06	0.52
730216	o.65	0.97	0.54	0.23
730217	0.41	0.52	0.55	0.27
730218	0.71	0.53	2.20	0.44
730219	0.55	0.60	0.52	0.37
730220	0.15	0.11	0.11	0.17
730221	0.54	0.40	0.42	0.46
730222	0.29	0.27	0.27	0.25
730223	0.20	0,24	0.18	0.31

730224	0.33	0.26	0.35	0.30
730225	0.53	0.40	1.12	0.54
730226	0.39	2.60	1.89	1.98
730227	0.40	0.38	1.03	1.34

Result: Out of 60 repeated aborted women 9 are IgM positive 51are IgG positive. From the above result we can conclude that the patients with IgM positive are recently infected with torch infections (American Academy of Pediatrics-2012)

IV. Discussion

The etiology of recurrent miscarriage (at least 9 consecutive miscarriages) is still a mystery. This study is mainly focused on immunological and genetic aspects of recurrent miscarriage. We determined IgG and IgM levels specific of TORCH group of infectious agents in women with recurrent miscarriage. (Cruse et. al.1995) Out of 60 samples, all were positive for TORCH profile and none were negative. 51 women had their serum positive for IgG levels and only 9 had their serum for IgM levels. IgG indicates past infection and IgM indicates recent infection.

The presence of TORCH group of infectious agents in the blood stream of infected pregnant women is considered deleterious as they lead to congenital anomalies or fetal loss (Cherry JD et.al; 2014). Therefore we found immunological alterations with regard to IgG and IgM levels in women with recurrent miscarriage. Next, vascular endothelial growth factor (VEGF) family of proteins and the receptors are vital for embryonic development (Xinx et.al; 2011). Therefore we determine VEGF gene polymorphisms (Rizk El-baz1 et.al; 2014) in women with a history of recurrent miscarriage. Apart from being a potent vascular endothelial mitogen,

VEGF maintains newly formed capillaries, induces vascular permeability and macrophage chemo taxis (Ferrara, 1999). It may thus give a novel aspect to the etiology of recurrent miscarriages. In tissues of uncomplicated pregnancy, we observed VEGF immuno reactivity in the placental trophoblasts as well as in the decidua, thus confirming previous findings. (Kraus RM et.al; 2004) However, the cyto- and syncytiotrophoblasts of women with MA or BO were negative for VEGF, giving support to the hypothesis of the role of VEGF (Vuorela P et.al; 2000) in the pathology of miscarriage.

In this study, a total of 60 women with Repeated Abortions were selected and an attempt was made to establish the association between VEGF 936C/T (Samli H et.al; 2012) polymorphism and Repeated Abortions. Several studies have suggested the role of VEGF in the pathophysiology of Repeated Abortions. VEGF has the ability to pass freely into the maternal circulation, increase cellular permeability and cell turnover and alter prostacyclin and NO production (Hayman et al., 1999). In the present study, we found 936C/T polymorphism. 12patients out of 60 are identified to have 936CT (Samli H et.al; 2012) allele and the remaining 48 patients have 936CC allele.

This may indicate that Repeated Abortion patients with normal homozygous genotype are more compared to the heterozygous genotype.

We did not find homozygous mutant genotype 936 TT among the repeated aborted patients of the present study. Shim et al 2007 have studied the association between +936 C/T polymorphism in Repeated Abortion. Our findings suggest further studies to be carried out in large number of samples for concrete information on the frequency and carriage of 936 C or T alleles in the susceptible women.

V. Conclusion

Our results indicate that recurrent miscarriage might be associated with immunological aberrations corresponding to TORCH group of infectious agents and VEGF 936 C/T polymorphisms. However, for concrete information on the role of infections and C/T allele's further studies are largely needed.

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